

1 WHAT IS CLAIMED IS:

1 1. A method for the vitrification of biological materials, said method comprising the
2 steps of:

3 (a) suspending the biological material in a cryoprotective equilibration medium,
4 having a concentration of cryoprotectant(s) below that sufficient to protect against
5 ice formation to the glass transition temperature of the cryoprotective equilibration
6 medium;

7 (b) rinsing the equilibrated biological material with a vitrification solution, the
8 vitrification medium having a concentration of cryoprotectant(s) sufficient to
9 protect against ice formation to the glass transition temperature of the vitrification
10 medium; and

11 (c) dropping the vitrification solution-rinsed biological material in microdroplets
12 of vitrification solution onto a solid surface with good heat conductivity having
13 been previously cooled down to about -150°C to about -180°C.

1 2. The method of claim 1 wherein the biological material is a cell.

1 3. The method of claim 1 wherein the biological material is an oocyte.

1 4. The method of claim 1 wherein the biological material is an embryo.

1 5. An improved method for cryopreserving biological material suspended in a
2 vitrification solution, wherein the improvement comprises contacting microdroplets of the
3 vitrification solution containing the biological material with a solid surface having a
4 temperature of about -150°C to about -180°C, said surface having a good heat
5 conductivity.

1 6. The method of claim 5 wherein the biological material is a cell.

1 7. The method of claim 5 wherein the biological material is an oocyte.

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1 8. The method of claim 5 wherein the biological material is an embryo.

1 9. An improved method for cryopreserving biological material suspended in a
2 vitrification solution, wherein the improvement comprises contacting microdroplets of the
3 vitrification solution containing the biological material with a solid surface having a
4 temperature of about -150°C to about -180°C , said surface having a thermal conductivity at
5 20°C of greater than about $10\text{ W}/(\text{m}\cdot\text{k})$.

1 10. A method for the vitrification of oocytes, said method comprising the steps of:

2 (a) suspending the oocytes in a cryoprotective equilibration medium, having a
3 concentration of cryoprotectant(s) below that sufficient to protect against ice
4 formation to the glass transition temperature of the cryoprotective equilibration
5 medium;

6 (b) rinsing the equilibrated oocytes with a vitrification solution, the vitrification
7 medium having a concentration of cryoprotectant(s) sufficient to protect against ice
8 formation to the glass transition temperature of the vitrification medium; and

9 (c) dropping the vitrification solution-rinsed oocytes in microdroplets of
10 vitrification solution onto a solid surface with good heat conductivity having been
11 previously cooled down to about -150°C to about -180°C .

1 11. An improved method of transferring nuclear DNA from a donor cell to an
2 enucleated oocyte, said improvement comprising the step of introducing the nuclear
3 material of the donor cell into an enucleated oocyte derived from an oocyte vitrified by the
4 method of claim 10.

1 12. An oocyte vitrified by the method of claim 10.

1 13. An embryo developed from the oocyte of claim 12.

1 14. A fetus developed from the oocyte of claim 12.

1 15. An animal developed from the oocyte of claim 12.

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- 1 16. A cell line developed from the embryo of claim 13.
- 1 17. A cell line developed from the fetus of claim 14.
- 1 18. A cell line developed from the animal of claim 15.
- 1 19. An improved method for cryopreserving oocytes suspended in a vitrification
2 solution, wherein the improvement comprises contacting microdroplets of the vitrification
3 solution containing the oocytes with a solid surface having a temperature of about -150°C
4 to about -180°C, said surface having a thermal conductivity 20°C of greater than about 10
5 W/(m-k).
- 1 20. An oocyte vitrified by the method of claim 19.
- 1 21. An embryo developed from the oocyte of claim 20.
- 1 22. A fetus developed from the oocyte of claim 20.
- 1 23. An animal developed from the oocyte of claim 20.
- 1 24. A cell line developed from the embryo of claim 21.
- 1 25. A cell line developed from the fetus of claim 22.
- 1 26. A cell line developed from the animal of claim 23.
- 1 27. An improved method for the parthenogenetic development of vitrified oocytes
2 cultured in a KSOM plus BSA culture system, the improvement comprising co-culturing
3 with cumulus-cells.

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